MRID: 49589501 **DP Barcode:** 426530

Data Evaluation Record for Thiamethoxam Field Study with Bumble Bee Colonies (*Bombus terrestris audax*)

Chemical: Thiamethoxam (**CAS #** 153719-23-4) **PC Code:** 060109
EPA Guideline: 850.3040
Test Materials: Seed treatment- Oil Seed Rape CABERNET treated with A9807F
Purity: Thiamethoxam[1] (420g/100kg trt seed – 0.03 mg a.i./seed[2])
CITATION: Wilkins, S. 2014. Thiamethoxam - Effects on Bumble Bee Colonies Foraging on Treated Oilseed Rape. Final Report Amendment 2

Study Completion Date: October 31, 2014

Sponsor: Syngenta Crop Protection, LLC, Greensboro, NC

Performing Laboratory: The Food and Environment Research Agency Centre for Chemical Safety and Stewardship Sand Hutton, York, UK
Study Number: W5ZT3002
Report Number: W5ZT3002
Task Number: TK0136490

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Date: 2016.06.10 15:25:18 -04'00'

Primary Reviewer: Ryan Mroz, Biologist **Date:** 6/1/2016
EPA/OPP/EFED/ERB1

Secondary Reviewer: Nicole McKenzie **Date:** 21 June 2016
Evaluation Officer, EAD, PMRA

Summary
The exposure phase was carried out using ~2 Ha fields with 25 bumble bee colonies: one field planted with 0.03 mg a.i./seed of thiamethoxam treated seed and two control fields planted with untreated seed. The 25 colonies were placed at the field edge (on-field) on each of the 3 test fields (C1, C2 and T1) when the crop was 20% in flower on the least developed field. The fields were drilled in autumn 2012. All agronomy and test item application data generated from the concurrent effects study. Colony development was assessed by monitoring the colony weight and activity during the exposure phase and daily assessments were made on bee activity within the crop. Once the exposure phase was completed (approximately 5 weeks - a total of 38 days from17/05/2013-21/06), the test colonies were moved from the exposure site to a site with a wide range of flowering plants that had not been exposed to neonicotinoids (post-exposure period was from 22/05/2013-17/07/2013).

Bombus terrestris were seen actively foraging within all three groups during the exposure period. Residues of both Thiamethoxam and the metabolite CGA322704 were found within the nectar

[1] The analyzed content per the analysis certificate was 466g/100kg of treated seed. These seeds were also treated with metalaxyl-M (57.7g/100kg trt seed), fludioxonil (13.4g/100kg trt seed). These residues were not quantified in the study report and are not considered further in this review.
[2] The 1000 seed weight is 6.239 g; 420 g a.i./100 kg seed (nominal) = 4.20 g a.i./ kg seed; 6.239 g/1000 seeds = 160,282 seeds/1 kg; Therefore: 4.20/160,282 *1000 = 0.03 mg a.i./seed

and pollen samples collected from the treated site. Colonies within all three treatment groups showed similar rates of average weight gain during the exposure phase. At the final weighing during the exposure phase the mean colony weight at site C1 was 626g, at site C2 639g and site T1 670g. There authors reported no treatment related colony failures (i.e. a total loss of bees or brood), and the mean number of queens produced per colony were similar between the three treatment groups (C1 (n=23) contained 18.6 (range 1 to 60), those on site C2 (n=21) 17.9 (range 1 to 67), with those on Tl (n=22) 21.3 (range 1 to 88)). The mean numbers of workers and drones produced by all colonies across the treatments was also very similar: average of 54, 47 and 58 workers for C1, C2, and T1 respectively and a mean of 33, 34 and 32 drones per colony in C1, C2, and T1 respectively. Some colonies on site T1 did appear to still be increasing in mass and had not started to produce queens about a week behind other colonies.

Materials and Methods:

Test organism

Bumble bees (*Bombus terrestris audax*) were obtained from the commercial supplier Biobest, Belgium N.V. *B. terrestris audax*. Each bumble bee colony was supplied with a queen and between 10-20 adult bees.

Experimental design (Exposure Phase – 17/05/2013-21/06/2013 (D0--D38))

The exposure phase was carried out using one thiamethoxam treated field (Treated – T1) and two untreated control fields (Control 1 and 2 – C1, C2) (located in Lincolnshire, UK) each of approximately 2 Ha. Twenty five bumble bee colonies per treatment group, were placed at the field edge (on-field) of the 3 test fields. The colonies were placed on the test fields when the crop on the least developed field (T1) was at 20% flowering and colony development (weight), and activity at entrance was recorded every 5-8 days (weather permitting). Daily assessments were made on bee activity within the crop by walking an approximated 100m transect over 10 minutes through a flowering section of the crop. After 38 days (exposure period) the colonies were removed to a monitoring site characterized by a wide range of flowering plants with no reported exposure to neonicotinoids. The colonies continued to be weighed until the weights started to decline – queen production phase (determined by when the majority of colonies had lost weight for two consecutive weeks). The colonies were returned to the laboratory and frozen to be assessed for numbers of workers, drones and queen bees produced as well as pupae, larvae and eggs present.

Field sites

The control fields and the treated fields were all drilled on October 6, 2012 at a nominal drilling rate of 4.25 Kg seed per Hectare (3.80 lb seed/acre = 0.0177 lb a.i/A[3]). This amounts to 0.03 mg a.i./seed. The three field sites were selected because it is not a major cropping area for oilseed rape (no other oilseed rape plants within ~5km) and were located on the Lincolnshire coast in the

[3] Based on analyzed content of 466 g a.i/100kg seed

UK. The predominant agriculture is intensive field vegetables such as cabbages, cauliflowers and potatoes (none of which flowered during the exposure period and where there is minimal neonicotinoid usage). There were no recorded applications of neonicotinoid compounds for the two cropping seasons prior to drilling of the test crops. The field sites were at least 5Km apart and were assigned to treatment group as follows (**Table 1**):

Table 1. Test Field Information

Site	Control 1	Control 2	Treated
Name	Wainfleet	Wrangle	Friskney
Location	0.298 858°E 53.092 422°N	0.162 735 °E 53.025 367°N	0.232 976°E 53.076 540°N
Size (m^2)	20170	20330	20130

Colonies

On Day 0 (17/05/2013) the numbers of bees per colony were counted and the total nest weight recorded. Nests with similar numbers of bees were grouped and then randomly allocated to fields. As colonies were placed in the field (at approx. 5 meter intervals), the entrances were restricted (7.5 mm diameter tube) to prevent newly emerged queens from leaving the colonies. The distance to the test crop was approximately 5 to 10 meters over a grassy track.

Plant, Pollen and Nectar sampling

Duplicate samples were collected during the course of the study, including whole plant, plant pollen and plant nectar samples for residue analysis of thiamethoxam and CGA322704. As part of study W5ZT3001, run concurrently with this study, samples of whole plants (pre-flowering and mid-flowering) and plant parts (flowers) were collected, additionally pollen and nectar samples were collected directly from the crop (details of samples collected may be seen in Appendix 4). All plant samples were collected on two occasions and stored frozen at approximately -10°C prior to return to the laboratory where they were stored frozen at a maximum of -20°C prior to analysis.

Pollen was collected by shaking the pollen from the stamens and nectar was collected using micro-capillary tubes (details of samples collected may be seen in Appendix 4). When the crop was in full flower (3 weeks), samples (approximately 200mg each) of pollen and nectar were removed from each of the nests at site Tl. Samples from five nests were combined to give a total of five samples of approximately 1g each of pollen and nectar. Pollen and nectar samples from sites C1 and C2 were collected after approximately four weeks in an identical manner, except that the combined sample from colony group C2/1 to C2/5 was from three colonies only (two had been accidentally destroyed by farm vehicles). Pollen samples were also analyzed using microscopic palynology techniques to identify the pollen collected (to at least Family to identify the plants upon which the bees had been foraging).

Each pollen grain was analyzed according to SOP NBU/ 057z using available reference books and material. Where possible each grain was identified to at least family level. Where grains of

the same type were identified a tally record was used. Similar looking grains which had the majority of the criteria matching, an estimated analysis was used, with a note 's.l.' after the scientific name to indicate identification was 'in the broader sense'. Where detailed identification was possible, the pollens were associated in larger family groups. Pollen grains that could not be matched and identified were recorded as 'unidentified.'

Post exposure phase and colony dissection (Post Exposure Phase – 22/05/2013-17/07/2013 (D39-D60))

After 5 weeks (38 days) the test crops were past full flowering and the colonies were placed in no particular order around the margin of a mixed field of cereals, soft fruits and grasses, including clover and weed covered waste ground with no neonicotinoid use in the immediate surrounding area in 2013. All colonies were kept on the post exposure monitoring site for 3 weeks and 2 days until the mean colony weights per treatment group had decreased – C1/C2 two weeks, T1 one week. There were an increasing number of colonies being destroyed by badgers so the T1 colonies were removed before weight decrease.

Colonies were removed from the freezer and dissected to assess the numbers of worker, queen and drone bees inside. The nest wax material was broken up to allow counting of eggs and young larvae and the numbers of queen (large) and worker/drone (smaller) pupae. Once counted, the nest material, minus the bees and eggs/larvae/pupae, was replaced into the nest cage and the unit re-weighed

Study Plan Deviations:
There were a number of deviations from the study plan reported by the authors. The following are a short summary:

1. Colony Weighing:
   The study plan stated that "all colonies will be weighed every 5-8 days." Due to poor weather this was carried out on one occasion after 9 days.

2. Activity Assessments:
   The study plan stated that "every 5-8 days the foraging activity at each colony will be assessed". This was delayed on one occasion to 8/9 days due to poor weather.

3. Foraging Assessments:
   The study plan stated that "The foraging activity of bees within the foraging crop will be recorded daily." On 24/05/13 the assessments were not carried out on any sites due to poor weather and on 29/05/13 the poor weather prevented the assessment being carried out on site Control 1 only.

4. Removal of colonies from the post exposure field site:
   The study plan states that "At least 8 weeks from the start of the trial (the exact time will be determined by the colonies having achieved maximum mass gain, at least 2 mass readings lower than the peak) the colonies will be weighed, returned to the laboratory and placed in the freezer." The decision was made to move the colonies from the field sites after 3 weeks and 2 days due to a number of nest boxes being destroyed by badgers. Out of the 66 colonies

that remained, 39 had 2 or more weights lower than the peak prior to being moved. The mean weights at sites C1 and C2 had 2 weeks of decreasing recordings prior to being moved – site T had 1 week of decreasing mean colony weight.

5. Initiation of agronomy phase:
The agronomy phase was started before this study was initiated under study W5ZT3001 (which was initiated on 28th September 2012). The same field sites were used for both studies and the field phase data are therefore shared between them. The raw data for the field phase will be held on the W5ZT3001 study file and certified copies will be held on the file for this study.

6. Start of flowering moving colonies to field sites:
The study plan stated that the hives would be moved onto the field sites when the least advanced field plot was at approximately 10% flowering. Following a discussion with the PI by telephone on the 10/05/13 the SD was informed that the all plots were coming into flower and that an estimation of the 10% flowering for the least advanced site (the treated plot) would be approximately sometime in the following week. Arrangements were made to move the bees onto the field site, at the time the bees were moved onto the field sites on the 16/05/13 and it was judged that at this time the treated plot had reached 20% flowering.

7. Appointment of Principle Investigator:
The study plan identified Thomas King, from Eurofins Agroscience Services Ltd as Principal Investigator for the Test Item Application and Agronomy. As all agronomy was carried out under GLP study W5ZT3001, appointment of the Principle Investigator were not required in this study. Certified copies of any raw data relevant to this study carried out by the Principle Investigator under study W5ZT3001 will be held on the raw data file for this study.

8. Reporting:
The study plan stated that the final report would include the study plan and all amendments. At the sponsors request this was not included. These deviations did not have any impact on the integrity or outcome of the study.

Results:

Colony Development
None of the colonies 'failed' (lost all queen and worker bees) during the study period, however, there were a number of losses due external physical factors:

- Two colonies at site C2 (C2/2 and C2/5) were accidentally destroyed when run over by a farm vehicle during week 4 and therefore data after week 4 was lost. The data up to that week were reported.
- Seven colonies (2 each from groups C1 and C2 [C1/19, C1/23 C2/7 & C2/8] and 3 from T1 [Tl/13, Tl/22 &T1/24]) were destroyed by badgers.

Generally, hives increased in weight (**Figure 1 -** from page 32 of study report) throughout the exposure. There was a slight decrease in hive weight the first 7 days after placement in the range

of 2 – 14 g followed by a steady increased rate until day 33. **Table 2** has the mean hive weights at several time points in the study.

After day 38, the colonies were moved off-site to the post-exposure monitoring locations. From day 47 onwards colonies from both sites C1 and C2 started to decline in mean weight. The mean weight gain for the treated group, T1 continued to increase until day 54. All hives were removed after day 61 (instead of after a full 2 weeks of decreased hive weight) to avoid continuing loss due to badgers.

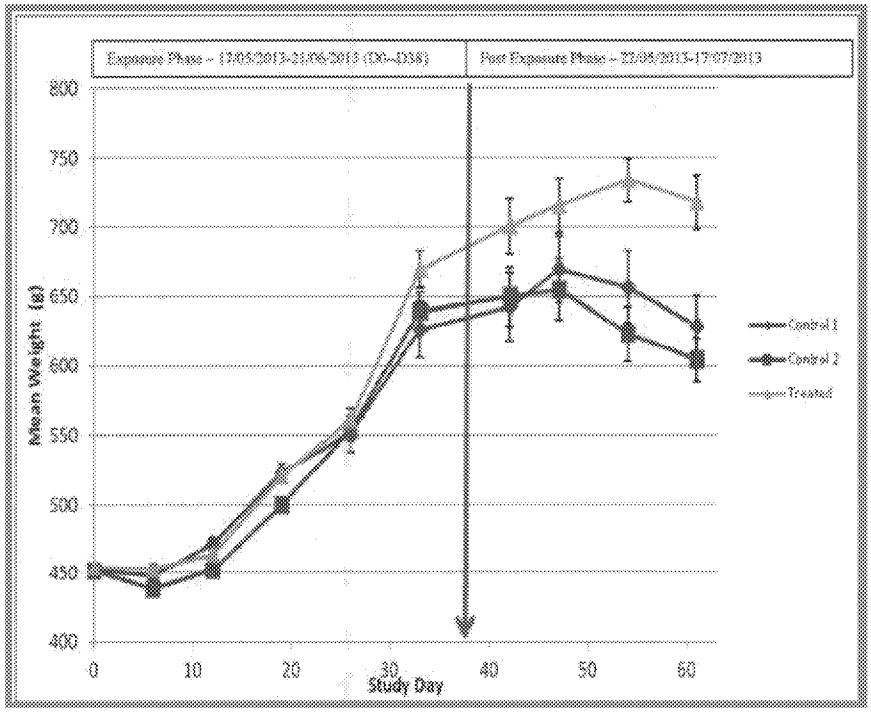

Figure 1. Mean colony weights throughout study (+/-SE)
Reviewer Note: It is presumed that the red arrow indicates when the exposure period ended after day 38 and the colonies were moved to their post-monitoring locations. Figure was taken directly from the study report.

Table 2. Hive weights at different time points of the study

TRT	Exposure start - 5/17 (± SD)	7 days post exposure - 5/23 (± SD)	Exposure end - 6/19 (± SD)	Peak Weight (± SD)	Termination - 7/17 (± SD)	At dissection (±SE)*
C1	453 ± 14	448. 5 ± 20	626 ± 126	670 ± 130 (7/3)	628 ± 117	560 ± 19.5
C2	451 ± 16	438 ± 30	639 ± 102	655 ± 95 (7/3)	604 ± 79	553 ± 14.1
T1	455 ± 18	453 ± 24	670 ± 99	734 ± 99 (7/10) **	718 ± 98	628 ± 14.7

SD = Standard Deviation
SE = Standard Error
*The authors reported the standard error rather than SD.
** Treatment weights peaked a week after control

Colony Activity

The authors report an increase in the mean levels of activity observed on all three sites during the observation period (**Figure 2** - from page 33 of the study report) there is overlap of the error bars for all treatments.

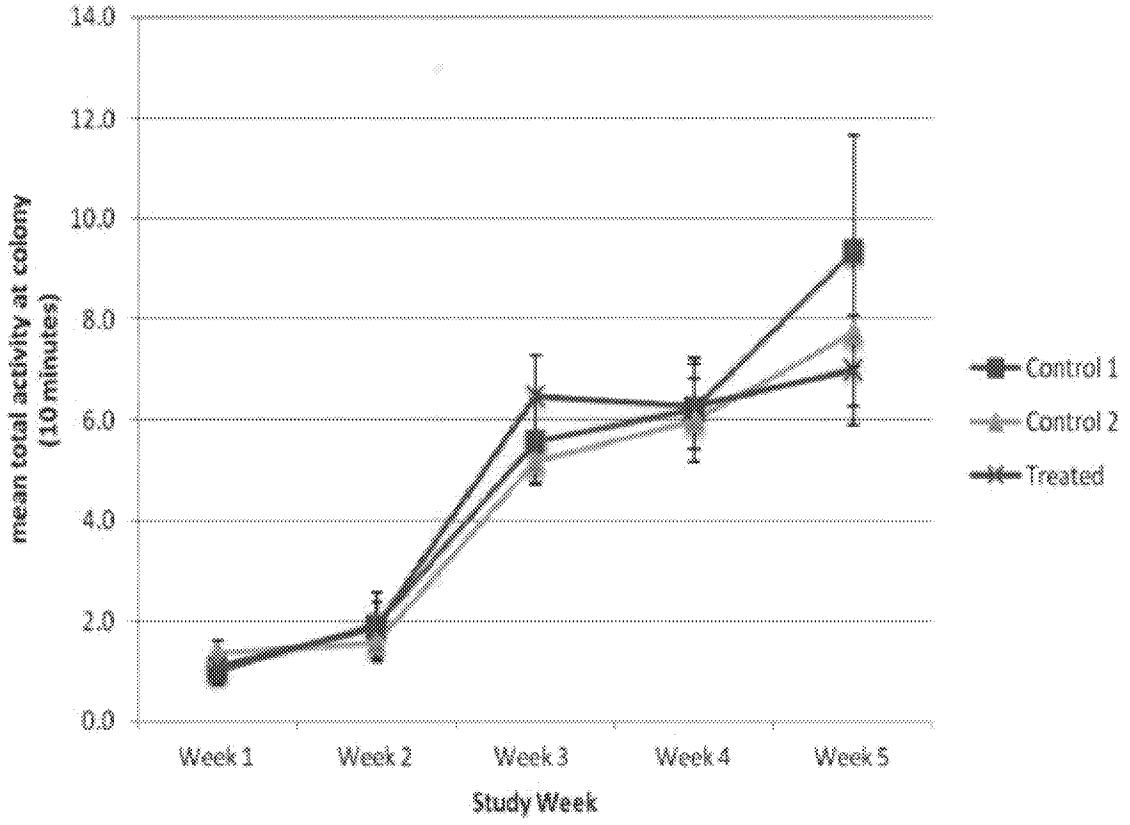

Figure 2: Mean total activity at colony entrance per 10 min observation period (Bees in and out) (±SE) Figure was taken directly from the study report.

During the colony weighings in the field a number of queens were observed between the cardboard nest box of the bumble bee colonies and the outer blue plastic box housing the colonies. The origin of these bees cannot be confirmed; these may have escaped from the test colonies and were unable to regain entry or originated from other study or non-study colonies. These were recorded as being present but were not included in authors the totals for queen production or the final analysis.

In Crop Activity

B. terrestris, and additional bee species, were active within the crop at all three field sites and the numbers of B. terrestris seen foraging on the crop increased over time (**Figure 3).** Not only did the amount of available forage on the fields increase but also the overall mean daily temperature increased through the study. The variation seen on a daily basis appears to be directly related to the local weather conditions, particularly rainfall and temperature

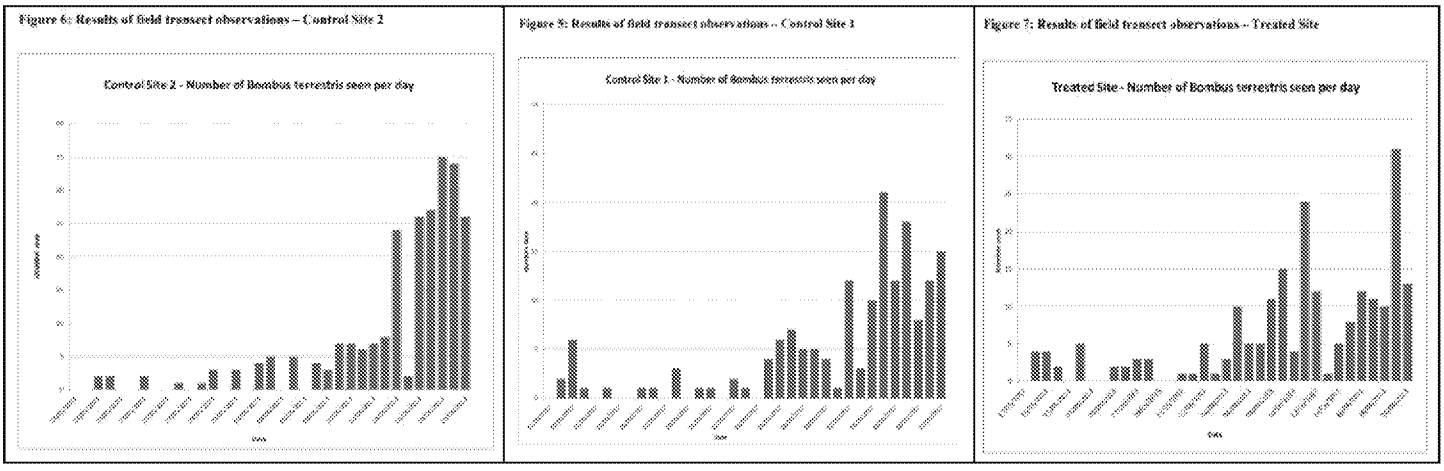

Figure 3. Foraging activity over time for the control and treatment sites. Figures taken directly from the study report.

Colony dissection

The details for the mean production of emerged adult bees (queens, drones and workers) as well as eggs, larvae (both in multi-occupancy (young) and single occupancy (older) cells) and pupae (small (worker/drones) and large (queens)) is presented in **Table 3 and Figure 4**.

Table 3. Summary of colony contents after dissection (+/-SE)

Treatment	Queens	Worker	Drones	Eggs	Total Larvae	Pupae Large	Pupae Small
C1	18.6 ± 3.5	54.4 ± 10.0	32.5 ± 5.3	34.9 ± 7.9	14.5 ± 4.1	3.9 ± 1.8	58.4 ± 10.3
C2	17.9 ± 4.0	47.2 ± 7.4	34.1 ± 6.6	21.0 ± 6.4	13.1 ± 5.0	1.5 ± 0.9	47.8 ± 7.0
T1	21.3 ± 5.0	57.7 ± 7.3	32.3 ± 5.4	66.0 ± 20.7	18.5 ± 5.7	5.4 ± 1.9	74.7 ± 10.0

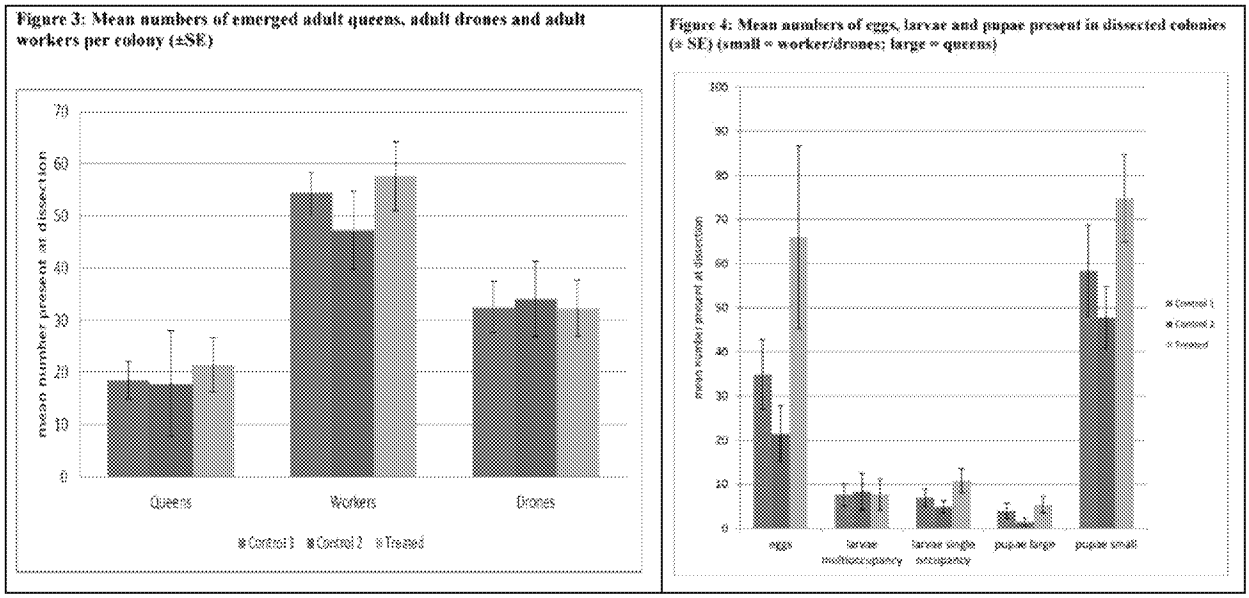

Figure 4. Mean numbers of various stages of brood and adults.

The mean total numbers of adult queens per colony are similar between the three treatment groups. The colonies on site C1 (n=23) contained 18.6 (range 1 to 60), those on site C2 (n=21) 17.9 (range 1 to 67), with those on T1 (n=21) 21.3 (range 1 to 88) (these figures include the foundress queen and therefore a colony with a single queen in would have produced no queens). The mean numbers of emerged workers and drones produced by all colonies across the treatments was also very similar with an average of 54, 47 and 58 workers for C1, C2 and T1 respectively and a mean of 33, 34 and 32 drones per colony in C1, C2 and T1 respectively.

The mean number of eggs, and large and small pupae in the treated colonies was higher than the control sites. The colonies from the treated site contained approximately double the mean numbers of eggs compared to the averages from the two control sites.

Residue analysis of crop collected samples

The Levels of Quantification (LOQ) for CGA322704 in all samples was 1.0 µg/kg and LOQ for Thiamethoxam in whole plants, flowers and pollen was 1.0 µg /kg and 0.5 µg /kg in nectar. *Reviewer Note: The limit of detection was not stated in this report.*

- Residues of CGA322704 (metabolite) were detected in one sample of pollen at 3.0 µg /kg (treated plot sample number 87, collected on 06/06/2013). Residues of Thiamethoxam were also detected in the same sample at 1.0 µg /kg.
- One nectar sample number (collected from the treated plot on 05/06/2013) was also shown to contain Thiamethoxam residues at a level of 1.8 µg /kg.

No residues of either Thiamethoxam or CGA322704 were detected in any of the samples of plants, flowers, pollen or nectar collected from the control fields at or above the level of quantification. These data were produced as part of Fera Study number W5ZT3001 and all raw data are held on the file for that study.

Palynological analysis of pollen nest collected samples

Within all of the treatment groups the highest proportion of pollen within the samples collected from the colonies was actually from oilseed rape (Brassica napus) –ranging from 73-92% (mean 84%) in Control 1, 45-91% (mean 68%) in Control 2 and 40-93% (mean 70%) in the treated group suggesting that the bumble bee colonies had actively foraged on the oilseed rape crop and that the majority of pollen taken back to the hive and forming part of the diet was comprised of oilseed rape. See Figure 5 below – taken directly from the study report.

Table 2: Summary of pollen identification - Control 1

	% pollen type from pooled pollen samples				
Pollen Family	**C1-1-5**	**C1-6-10**	**C1-11-15**	**C1-16-20**	**C1-21-25**
Compositae	-	5	-	-	-
Cruciferae (OSR)	76	85	73	93	92
Liliaceae	1	2	-	-	-
Plantaginaceae	4	1		-	-
Rosaceae	9	1	8	-	5
Salicaceae (Salix)	-	-	-	-	3
Umbelliferae	8	1	16		
Unidentified	2	5	3	7	
Total	100	100	100	100	100

Table 3: Summary of pollen identification - Control 2

	% pollen type from pooled pollen samples				
Pollen Family	**C2-1-3-4**	**C2-6-10**	**C2-11-15**	**C2-16-20**	**C2-21-25**
Aceraceae	1	4	3	-	-
Aquifoliaceae	12	15	6	6	2
Compositae		3			
Cruciferae (OSR)	67	69	45	66	91
Papilionaceae			1	6	-
Rosaceae	16	4	17	8	2
Salicaceae (Salix)	-	3	20	2	-
Umbelliferae	-	-	5	11	1
unidentified	4	2	3	1	4
Total	100	100	100	100	100

Table 4: Summary of pollen identification - Treated

	% pollen type from pooled pollen samples				
Pollen Family	**T1-1-5**	**T1-6-10**	**T1-11-15**	**T1-16-20**	**T1-21-25**
Aceraceae	-	5	-	2	-
Aquifoliaceae	7	4	5	2	5
Cruciferae (OSR)	93	69	79	71	40
Papilionaceae	-	-	-	-	2
Plantaginaceae	-	-	-	-	7
Rosaceae	-	-	-	-	22
Umbelliferae	-	21	16	21	18
Unidentified	-	1	-	4	6
Total	100	100	100	100	100

Figure 5. Pollen Identification in the controls and treated seed group

Conclusions

Colonies within all three treatment groups showed similar rates of average weight gain during the exposure phase, up to day 33. The rate of weight gain for colonies from site C2 slowed in relation to those from sites C1 and T1 after being moved to the post exposure site.

Some colonies on site T1 did appear to still be increasing in mass and had not started to produce queens and were in the region of a week behind other colonies. The mean number of eggs, and large and small pupae in the treated colonies was higher than the control sites by the end of the experiment. The colonies from the treated site contained approximately double the mean numbers of eggs compared to the averages from the two control sites.

Study Limitations: Only one treated field two control field were tested each year (i.e., one replicate with 25 hives – repeated measures – for the treatment). The slight decrease in mass 7 days after placement may indicate some stress from transport and exposure, but appears to be transient. Some colonies on site T1 did appear to still be increasing in mass and had not started to produce queens and were in the region of a week behind other colonies. It is unclear if this could be attributed to a delay in colony development due to the treatment or natural variation. During the colony weighings in the field a number of queens were observed between the cardboard nest box of the bumble bee colonies and the outer blue plastic box housing the colonies. The origin of these bees cannot be confirmed; these may have escaped from the test colonies and were unable to regain entry or originated from other study or non-study colonies. These were recorded as being present but were not included in authors the totals for queen production or the final analysis.

On its own this study does not provide enough adequate information on residues; however, in combination with the concurrently run effects study may provide enough information to quantify residues in bumble bee pollen and nectar. The limit of detection was not stated in this report.

Agency Response: This study is classified as "**Supplemental (qualitative)**" because of the uncertainties regarding the methodology (see the Study Limitations section). It can be useful as a weight of evidence when characterizing risks of thiamethoxam and CGA322704 on bumble bee colonies when exposed to seed treated with thiamethoxam. Combined with the results from the other (concurrently run) effects study conducted, it may show patterns of effects to bumble bees that are exposed to seed treated with thiamethoxam.

Peer Review Response: The PMRA agrees with the classification of **qualitative (informative)** for this study. See reviewer comments throughout the DER and in the Conclusion section for more details.

Additional Reviewer Comments:

This study was conducted concurrently with a honeybee effects study which details the agronomy and test item application data generated from that study: Thiamethoxam – Effects on homing behaviour of honeybees foraging on treated oilseed rape. Author: Selwyn Wilkins. Report Date: 2014-October-31.

These seeds were also treated with metalaxyl-M (57.7g/100kg trt seed), fludioxonil (13.4g/100kg trt seed). These residues were not quantified in the study report and are not considered further in this review.